The resistance of Varroa jacobsoni Oud. to acaricides

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Review article

The resistance of *Varroa jacobsoni* Oud. to acaricides

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Abstract – A serious problem in the control of the mite *Varroa jacobsoni* Oudemans is the selection of acaricide-resistant strains. Increased tolerance of this mite to several active substances (acrinathrin, amitraz, bromopropylate, chlordimeform, coumaphos, flumethrin, fluvalinate) was determined with laboratory assays or presumed as the most likely explanation of decreased field efficacy. Resistance to fluvalinate in Italy and later in other countries caused heavy damage to beekeeping. Early detection of the presence of resistant *Varroa* mites was crucial to reduce losses. Regarding resistance management tactics, 'moderation tactics' seem to be more suitable than 'high dose tactics' to delay the selection of resistant *Varroa* strains. © Inra/DIB/AGIB/Elsevier, Paris

***Varroa jacobsoni* / honey bee / resistance / acaricide / fluvalinate**

1. INTRODUCTION

Controlling the parasitic mite, *Varroa jacobsoni* Oudemans (Mesostigmata: Varroidae), became easier during the late 1980s with the increased availability of acaricide products that were very effective, tolerated by the honey bee (*Apis mellifera* L.) and easy to use [8]. Acaracides seemed to offer a solution to the problem caused by the mite. Although researchers and beekeepers were aware of the limits of this approach – the build-up of residues and the selection of resistant *V. jacobsoni* strains – an appreciation of the limits only became evident in recent years. In particular, the onset of resistance to acaricides has caused serious concern because only a small number of compounds are suitable for mite control, and thus the problem is not easily overcome by changing the active ingredient used.

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2. INCREASED TOLERANCE TO DIFFERENT ACARICIDES

The existence of populations of *V. jacobsoni* showing an increased tolerance to the active ingredients used for the control of this mite has been reported for several widely used acaricides.

2.1. Amitraz

Inefficacy of amitraz in Vojvodina, a region of the former Yugoslavia, after 4 years of successful use, was reported [4]; the most likely explanation was the presence of resistant mites. Little attention was paid to this problem since amitraz was substituted with fluvalinate.

2.2. Bromopropylate

Ritter and Roth [23] described a gradual increase in the LD_{50} for mites taken from small colonies (nuclei) repeatedly treated with low doses of this active ingredient.

2.3. Chlordimeform

An increase in the LD_{50} under similar conditions as bromopropylate was also reported [23].

2.4. Coumaphos

Since the spread of *V. jacobsoni* mites resistant to fluvalinate, the organophosphorous acaricide, coumaphos, has been widely used for the control of *V. jacobsoni* in Italy and in nearby countries. In 1995, field trials carried out in an area in northern Italy showed that the efficacy of the commercial formulation Perizin, containing coumaphos as the active ingredient, had decreased to 36–80 % in several apiaries [12]; this raised much concern about the possible onset of resistance. In 1996, laboratory assays [9, 18] showed a slight (2–3 fold), but significant increase in the tolerance to coumaphos of samples of mites taken from that region; the mortality at LC_{95} (determined for susceptible mites) decreased to about 50 %. Coumaphos was no longer used to control *V. jacobsoni* in that area. One year later, samples of mites taken in adjacent areas and in other regions did not show any increase in the tolerance of *V. jacobsoni* to coumaphos (Milani and Della Vedova, unpublished data). On the other hand, in Italy this acaricide was widely substituted with oxalic acid [19] in autumn treatments, and thus the problem lost part of its urgency.

2.5. Fluvalinate

The resistance to fluvalinate in Italy had a much more serious impact on beekeeping and was better studied. In 1991–1992 reduced efficacy of Apistan (active ingredient: fluvalinate) was reported from Lombardy [13, 14], a region of northern Italy where the veterinary services had given free Apistan strips to all the beekeepers. The selection pressure from regular treatments with Apistan strips was evidently sufficient to select resistant mites. Similar problems seem to have occurred in Sicily, but precise data are lacking. Although these Italian regions are about 1 000 km apart, they are connected by active migratory beekeeping.

The consequences were disastrous colony losses, exceeding 50 % and in some locations up to 90 % in districts where statistics are available [22]. These losses were usually unexpected since no abnormal increase in *V. jacobsoni* infestation or unsatisfactory efficacy of treatments had been observed during the previous years. The presence of extremely high infestation levels clearly indicated the cause of the problem, but this was not readily recognized [26]. The resistant *V. jacobsoni* mites spread to all regions of Italy very quickly owing to the high selection pressure (nearly all the colonies, except feral swarms, over all the country were treated with fluvalinate). Later the fluvali-

nate-resistant strain crossed the Alps – both a geographical and political barrier – and was detected in nearby countries [25]. The distribution of the resistant mites was initially rather patchy; higher proportions of resistant mites were usually recorded in areas where migratory beekeeping was more active [17] indicating its primary role in the spread of the resistant strain.

A laboratory assay [15, 16] confirmed that the inefficacy of Apistan was due to the presence of fluvalinate-resistant *V. jacobsoni* mites. This strain of mites showed cross resistance between fluvalinate and two closely related pyrethroids (flumethrin and acrinathrin). The bioassay was validated by extensive examination of European populations of *V. jacobsoni*, both susceptible and resistant [25], and was used routinely to monitor the spread of resistant populations in Italy and Europe [25]. Other laboratory and field assays were developed later [3, 6, 21]. Early detection of the presence of resistant mites was crucial in reducing the economic damage [17].

The biochemical mechanism of the resistance is only partly understood; although other factors could play a role and the resistance could be a polygenic character, it has been shown that the monooxygenases of the P450 system – the detoxicating enzymes that make fluvalinate almost harmless to the bee – are involved [11]. The use of inhibitors of monooxygenases, such as the piperonyl butoxyde, as synergics would make fluvalinate toxic to the bee.

Resistance in insects or mites is often associated with lower fitness [10] and this leads to the decline in the frequency of the alleles for resistance (reversion) when the active ingredient is not used. In the case of the Italian strain of *V. jacobsoni* resistant to fluvalinate, the first data (Trouiller, pers. comm.; Milani and Della Vedova, unpublished data) showed a slow decrease in the proportion of resistant mites.

More recently, unsatisfactory control of *V. jacobsoni* with Apistan, caused by the presence of mites resistant to fluvalinate, was recorded in Florida and in other states of the USA [1, 5].

3. RESISTANCE MANAGEMENT

Theoretical and empirical work has been carried out to develop tactics to delay the development of resistance of insects or mites (reviewed in [10]). 'High dose tactics' aim at eliminating the most resistant alleles by using a high enough dose to kill the heterozygotes, who are supposedly endowed with a lower level of resistance. A 'high dose tactic' would thus appear to be less appropriate for the *V. jacobsoni* mite, whose populations are highly inbred and show a high degree of homozygosity [2]. However, this tactic could still be valid if mutations giving a higher degree of resistance were less frequent or if resistance were polygenic.

In the case of *V. jacobsoni*, tactics meant to preserve susceptible mites ('moderation tactics') by avoiding excessive mite kill, could be more suitable for slowing the selection of resistant mites. The success of these tactics depends on the balance between the selection pressure resulting from the application of acaricides and disadvantages associated with resistance, which would cause the frequency of genes for resistance to decrease in the interval between treatments. The disadvantage associated with resistance is very variable and often small (reviewed in [24]). In the case of the *V. jacobsoni* mite, however, even a decrease in the fitness in the order of a few percent per generation would produce an appreciable disadvantage over 1 year, since several generations of the mite take place during this period. On the other hand, the selection pressure increases dramatically when the efficacy of treatments approaches 100 % and the same acaricide is used repeatedly or for prolonged periods, reducing correspondingly the effect of any disadvantage of the resistant strain.

To maintain the control of the mite with treatments having a reduced efficacy,

chemotherapy could be integrated with non-chemical control techniques and different acaricides could be applied in different seasons of the year, each one acting for a restricted period of time. For example, since the spread of fluvalinate-resistant *V. jacobsoni* strains, many Italian beekeepers control this mite with a combination of ethereal oils at the end of summer and a single treatment with an organophosphorus acaricide or oxalic acid in late autumn. Another option would be to use different active ingredients in successive years. Treatment schedules based on rotation of acaricides from non-cross-resisted groups have been proposed for different mites pest of orchards [e.g. 20] and their potential has been assessed in field experiments [e.g. 7].

These measures could be coupled with the selection of bee strains on which *V. jacobsoni* populations increase at a slower rate, thus making highly effective treatments unnecessary and delaying the selection of resistant mite strains.

Résumé – La résistance de *Varroa jacobsoni* Oud. aux acaricides. Des acaricides efficaces contre l'acarien *V. jacobsoni* ont été disponibles dans les années 80, mais rapidement on a signalé des échecs de traitement, que l'on pensait être provoqués par la résistance à des matières actives largement employées. Des tests au laboratoire ont mis en évidence une tolérance accrue de l'acarien à diverses matières actives utilisées contre lui (bromopropylate, chlordiméforme, coumaphos, fluvalinate et deux pyréthrinoïdes très proches, l'acrinathrine et la fluméthrine). La résistance à l'amitraz a été considérée comme l'explication la plus plausible de la diminution d'efficacité au champ, mais n'a pas été testée au laboratoire. La résistance au fluvalinate, l'acaricide le plus utilisé dans la lutte contre *V. jacobsoni*, a été détectée en premier lieu en Italie (1992–1993) [13], puis dans d'autres pays européens [25] et récemment aux États-Unis [1, 5]. Elle a provoqué de fortes pertes de colonies, auxquelles en général on ne s'attendait pas car on n'avait pas observé dans les années précédentes d'augmentation anormale de l'infestation ni une moindre efficacité des traitements. Un test au laboratoire a été utilisé en routine pour suivre la progression des souches résistantes en Europe [25]. Le mécanisme biochimique de la résistance au fluvalinate n'est que partiellement compris ; on a montré que les monooxygénases du système P450 étaient impliquées [11]. En ce qui concerne la tactique à suivre vis-à-vis de la résistance au fluvalinate, la tactique modérée, qui fait preuve d'une confiance réduite en un seul traitement chimique répété, semble mieux convenir que la tactique de forte dose pour retarder la sélection de souches de *V. jacobsoni* résistantes.

***Varroa jacobsoni* / abeille mellifère / résistance / acaricide / fluvalinate**

Zusammenfassung – Die Akarizidresistenz von *Varroa jacobsoni*. Während der achtziger Jahre wurden wirksame Akarizide zur Bekämpfung von *Varroa jacobsoni* verfügbar. Bereits nach verhältnismäßig kurzer Zeit wurde über Fehlschläge dieser Behandlungen berichtet; für diese wurde das Entstehen von Resistenzen gegenüber diesen weithin gebräuchlichen aktiven Substanzen verantwortlich gemacht. Die Empfindlichkeit von *Varroa jacobsoni* gegenüber mehreren zur Bekämpfung der Varroose eingesetzten Substanzen (Brompropylat, Chlordimeform, Coumaphos, Fluvalinat und zwei weitere nah verwandte Pyrethroide, Acrinathrin und Flumethrin) wurde in Laboruntersuchungen ermittelt. Resistenz gegenüber Amitraz wurde als die wahrscheinlichste Erklärung für die abnehmende Wirksamkeit im Freiland angesehen, diese Substanz wurde allerdings bisher nicht im Labor untersucht. Resistenz gegenüber Fluvalinat, das gebräuchlichste Bekämpfungsmittel gegen *V. jacobsoni*, wurde zunächst in

Italien und später auch in einigen weiteren Ländern festgestellt. Dies führte zu schweren und unerwarteten Verlusten an Bienenvölkern, da in den Vorjahren meist weder ein außergewöhnliches Anwachsen der Varroapopulationen noch eine unbefriedigende Wirksamkeit der Bekämpfung beobachtet worden waren. Zur Vermeidung von Verlusten ist eine frühzeitige Erkennung des Auftretens resistenter Milbenlinien daher sehr wesentlich. Es wurde routinemäßig ein standardisierter Test eingesetzt, um das Ausbreiten resistenter Milben in Europa verfolgen zu können. Die biochemische Grundlage der Resistenz gegenüber Fluvalinat ist bisher nur zu Teilen verstanden, offensichtlich sind hierbei die Monooxigenasen des P450-Systems beteiligt. Zum Umgang mit der Resistenz erscheinen *moderation tactics* mit einer Verminderung der Abhängigkeit von einem wiederholten Einsatz einer einzigen Chemikalie eher angemessen als *high dosage tactics*, mit denen versucht wird, die Herausbildung resistenter Varroalinien zu verzögern.

***Varroa jacobsoni* / Honigbienen / Resistenz / Akarizide / Fluvalinat**

REFERENCES

[1] Baxter J., Eischen F., Pettis J., Wilson W.T., Shimanuki H., Detection of fluvalinate-resistant varroa mites in U.S. honey bees, Am. Bee J. 138 (1998) 291.

[2] Biasiolo A., Lack of allozyme variability among *Varroa* mite populations, Exp. Appl. Acarol. 16 (1992) 287–294.

[3] Colin M.E., Vandame R., Jourdan P., Di Pasquale S., Fluvalinate resistance of *Varroa jacobsoni* Oudemans (Acari: Varroidae) in Mediterranean apiaries of France, Apidologie 28 (1997) 375–384.

[4] Dujin T., Jovanović V., Šuvakov D., Milković Z., Effects of extended utilisation of amitraz-based preparations on the formation of resistant strains of *Varroa jacobsoni*, Vet. Glas. 45 (1991) 851–855 (in Croatian (latin script), with Serbian spelling).

[5] Elzen P.J., Eischen F.A., Baxter J.R., Pettis J., Elzen G.W., Wilson W.T., Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations, Am. Bee J. 138 (1998) 674–676.

[6] Faucon J.P., Drajnudel P., Fléché C., Varroose : mise en évidence de la résistance du parasite aux acaricides par la méthode de « détermination du temps létal moyen », Apidologie 27 (1996) 105–110.

[7] Flexner J.L., Westigard P.H., Hilton R., Croft B.A., Experimental evaluation of resistance management for twospotted spider mite (Acari: Tetranychidae) on Southern Oregon Pear, J. Econ. Entomol. 88 (1995) 1517–1524.

[8] Koeniger N., Fuchs S., Eleven years with *Varroa* – experiences, retrospects and prospect, Bee World 70 (1989) 148–159.

[9] Della Vedova G., Lodesani M., Milani N., Resistenza di *Varroa jacobsoni* al coumaphos?, L'ape nostra amica 19 (1) (1997) 6–10.

[10] Denholm I., Rowland M.W., Tactics for managing pesticide resistance in arthropods: theory and practice, Annu. Rev. Entomol. 37 (1992) 91–112.

[11] Hillesheim E., Ritter W., Bassand D., First data on resistance mechanisms of *Varroa jacobsoni* (Oud.) against tau-fluvalinate, Exp. Appl. Acarol. 20 (1996) 283–296.

[12] Lodesani M., Variabilità dell'efficacia terapeutica ottenuta con trattamento di Perizin, L'ape nostra amica 18 (5) (1996) 5–9.

[13] Lodesani M., Colombo M., Spreafico M., Ineffectiveness of Apistan® treatment against the mite *Varroa jacobsoni* Oud in several districts of Lombardy (Italy), Apidologie 26 (1995) 67–72.

[14] Loglio G., Plebani G., Valutazione dell'efficacia dell'Apistan, Apic. mod. 83 (1992) 95–98.

[15] Milani N., Possible presence of fluvalinate-resistant strains of *Varroa jacobsoni* in northern Italy, in: Matheson A. (Ed.), New Perspectives on Varroa, Proc. Int. Meet., Prague, November 1993, IBRA, Cardiff, 1994, p. 87.

[16] Milani N., The resistance of *Varroa jacobsoni* Oud. to pyrethroids: a laboratory assay, Apidologie 26 (1995) 415–429.

[17] Milani N., Die Ausbreitung apistanresistenter Varroamilben im nordöstlichen Italien, Bienenvater 117 (1996) 290–193.

[18] Milani N., Della Vedova G., Determination of the LC50 in the mite *Varroa jacobsoni* of the active substances in Perizin® and Cekafix®, Apidologie 26 (1996) 67–72.

[19] Nanetti A., Stradi G., Varroasi: trattamento chimico con acido ossalico in sciroppo zuccherino, L'ape nostra amica 19 (5) (1997) 6–14.

[20] Pree D.J., Inheritance and management of cyhexatin and dicofol resistance in the European red mite (Acari: Tetranychidae), J. Econ. Entomol. 80 (1987) 1106–1112.

[21] Pettis J.S., Shimanuki H., Feldlaufer M.F., An assay to detect fluvalinate resistance in *Varroa* mites, Am. Bee J. 138 (1998) 538–541.

[22] Regione Lombardia, Le disposizioni annuali per una corretta lotta alla varroasi, L'ape nostra amica 17 (3) (1995) 10–17.

[23] Ritter W., Roth H., Experiments with mite resistance to varroacidal substances in the laboratory, in: Cavalloro R. (Ed.), European research on varroatosis control, Proc. Meet. EC Experts' Group, Bad Homburg, October 1986, Balkema, Rotterdam, 1988, pp. 157–160.

[24] Roush R.T., Daly J.C., The role of population genetics in resistance research and management, in: Roush R.T., Tabashnik B.E. (Eds.), Pesticide Resistance in Arthropods, Chapman and Hall, New York, 1990, pp. 97–152.

[25] Trouiller J., Monitoring of *Varroa jacobsoni* resistance to pyrethroids in Europe, Apidologie 29 (1998) 537–546.

[26] Watkins M., Resistance and its relevance to beekeeping, Bee World 77 (4) (1996) 15–22.